Biochemical and Biophysical Research Communications 430 (2013) 1109–1113

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Heat shock protein 90 (HSP90) inhibitors activate the heat shock factor 1 (HSF1) stress response pathway and improve glucose regulation in diabetic mice

Jee-Hyung Lee, Jiaping Gao, Penelope A. Kosinski [4], Stephen J. Elliman [1], Thomas E. Hughes [2], Jesper Gromada [3], Daniel M. Kemp *

Cardiovascular and Metabolism Disease Area, Novartis Institutes for Biomedical Research, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 4 December 2012
Available online 19 December 2012

Keywords:
HSP90
HSP70
Type 2 diabetes
Pharmacology

ABSTRACT

The cytoprotective stress response factor HSF1 regulates the transcription of the chaperone *HSP70*, which exhibits anti-inflammatory effects and improves insulin sensitivity. We tested the therapeutic potential of this pathway in rodent models of diabetes using pharmacological tools. Activation of the HSF1 pathway was achieved using potent inhibitors of the upstream regulatory protein, HSP90. Treatment with AUY922, a selective HSP90 inhibitor led to robust inhibition of JNK1 phosphorylation, cytoprotection and improved insulin signaling in cells, consistent with effects observed with HSP70 treatment. Chronic dosing with HSP90 inhibitors reversed hyperglycemia in the diabetic *db*/*db* mouse model, and improved insulin sensitivity in the diet-induced obese mouse model of insulin resistance, further supporting the concept that the HSF1 pathway is a potentially viable anti-diabetes target.

1. Introduction

All organisms are susceptible to intracellular stress and are thus predisposed to stress-induced metabolic dysfunction. For example, increased reactive oxygen species, protein misfolding, and DNA damage accumulate to varying degrees across species, tissues and cell types and are influenced by both genetic and environmental pressures, including age. In the context of metabolic diseases, such as type 2 diabetes, intracellular stress pathways are tightly linked to nutrient sensing and metabolic mechanisms and these integrated pathways coordinately govern metabolic functions such as insulin sensitivity and glycemic state [1–5]. Studies in rodents reveal that genetic and pharmacological intervention of cellular stress pathways can effectively relieve metabolic dysfunction [3,6]. For example, inhibition of the stress-inducible c-jun amino terminal kinase (JNK) pathway in obese diabetic mice, or genetic deletion of JNK1, markedly improves insulin resistance and ameliorates glucose intolerance [7]. The mechanistic rationale for this efficacy is that JNK1 directly phosphorylates IRS-1 at inhibitory serine residues, causing attenuation of the insulin signaling pathway in several metabolic tissues [8,9]. Notably, IRS-1 ser-307 phosphorylation is markedly increased in obese mice, although no such increase is observed in obese JNK1$^{-/-}$ mice that are protected from insulin resistance [10]. In addition, JNK1 inhibitors have been shown to block TNFα-induced IRS-1 ser-307 phosphorylation in cell culture models of insulin resistance further demonstrating the coordination of stress and nutrient sensing pathways [7].

Besides targeting specific downstream genes of stress pathways (such as JNK1) to alleviate insulin resistance, an alternative approach is to activate endogenous protective stress response pathways, which may impart greater efficacy [11]. We tested this hypothesis by targeting the HSF1 (heat shock factor 1) pathway, an evolutionarily conserved transcriptional stress response program comprised of a large repertoire of cytoprotective chaperone genes [12]. HSF1 exists in the cell under tight negative regulation by a multi-subunit complex that includes HSP90, a ubiquitous and highly abundant chaperone. Evidence that HSP90 specifically represses HSF1 emerged from observations that selective HSP90 inhibitors were potent activators of HSF1-dependent transcription [13]. We therefore employed HSP90 inhibitors to assess whether the HSF1 pathway may effectively counteract cellular stress and metabolic dysfunction in both cell culture and *in vivo*. Furthermore, we tested whether activation of this stress response can improve glycemic control in diabetic (*db*/*db*) mice. Finally, we focused on potential molecular mediators of the cellular and metabolic effects of HSP90 inhibitors such as JNK1, IRS1 and AKT phosphorylation.

* Corresponding author. Present Address: Merck Research Laboratories, 126 East Lincoln Ave., Rahway, NJ, USA.
E-mail address: daniel_kemp@merck.com (D.M. Kemp).
[1] Current address: Orbsen Therapeutics, NUI Galway, Ireland.
[2] Current address: Zafgen Inc., One Cambridge Center, Cambridge, MA, USA.
[3] Current address: Regeneron Pharmaceuticals, Tarrytown, NY, USA.
[4] Current address: Agios Pharmaceuticals, Cambridge, MA, USA

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.029

2. Materials and methods

2.1. In vitro studies

Primary mouse skeletal myoblasts were passaged and maintained on collagen-1 coated tissue culture plastics (BD BIOCOAT™) at low density (2–8 × 10^3 cells/cm^2) prior to initiation of differentiation to prevent spontaneous differentiation. To obtain differentiated myotubes, cells were grown to confluence in growth media and upon confluence cells were switched to a low serum differentiation media (high glucose DMEM, 2% horse serum, Invitrogen). Mature myotube differentiation was completed within 48 h of initiation. For dose response studies, geldamycin (GA) and 17-AAG concentrations ranged from 100 nM to 10 μM. AUY922 concentration ranged from 1 nM to 10 μM. DMSO vehicle concentration was maintained at 0.1%. Treatment lasted for 5 h and cells were harvested in RIPA buffer.

To induce insulin resistance in C2C12 cells, myoblasts were cultured in DMEM medium containing 15 g/L glucose, 10% FBS, 1 μg/ml insulin, and 100 nM dexamethasone for 3 days. This medium was used for the normal passaging procedure. Cells were serum starved for 2 h prior to insulin stimulation and when appropriate, AUY922 in 0.1% DMSO final concentration was added to the serum-deficient media concurrently. Cells were then stimulated with 100 nM insulin for 15 min prior to harvest.

INS-1E cells were cultured in RPMI-1640 supplemented with 10% heat inactivated FBS, 10 mM HEPES, 1 mM sodium pyruvate, 50 μM beta-mercaptoethanol, 1% pen/strep, and seeded at 10^4 cells/well in white-walled 96 well plates. Two days after plating, cells were placed in serum free RPMI-1640 media with 0.5% fatty acid free BSA for 2 h. Following serum deprivation, cells were exposed to RPMI-1640 media containing 5% FBS, 0.5% BSA, mouse IL-1β and mouse IFNγ (4 ng/ml each). Cells were then incubated overnight (~18 h) with AUY922. Promega's Apo-ONE Homogeneous Caspase 3/7 assay kit was used according to the manufacturer's directions to determine apoptosis, and measurements were conducted on a Victor3 plate reader.

HSP70 antibody (SPA-810) was purchased from Stressgen. The tubulin antibody (ab3194) was purchased from Abcam. The pAkt S473 (#4058), total Akt (#9272), pJNK Thr183 (#9251), and total JNK (#9252) antibodies were purchased from Cell Signaling.

2.2. In vivo studies

Male BKS.Cg-*m Lepr db*/++ mice (*db*/*db* mice, Jackson Lab, Bar Harbor, ME) at age 6 weeks were housed two per cage in a reversed light cycle room (light on from 8:00 p.m. to 8:00 a.m.) and given access to Purina rodent chow and water ad libitum. The animals were used for the study from 7 weeks of age. For studies with diet-induced obese (DIO) C57B6 mice, animals were fed a high fat diet (D12492 with 60% caloric intake from fat, Research Diets, New Brunswick, NJ) from 6 weeks of age and were maintained on the diet throughout the study starting at 16 weeks of age. All procedures in this study were in compliance with the Animal Welfare Act Regulations 9 CFR Parts 1, 2 and 3, and other guidelines. Blood samples were taken by tail vein bleeding, collected in EDTA (ethylene diaminetetraacetic acid)-coated tubes and kept on ice. Plasma was obtained by centrifugation of blood at 10,000*g* for 10 min at 4 °C and stored at −80 °C. Animals were randomly assigned into vehicle and compound groups (n = 8/group) with the mean plasma glucose level and body weight matched among the groups. Following body weight measurement, animals were dosed in the morning via tail vein injection (5 ml/kg) with saline or AUY922 in saline. At 8, 24 and 48 h post dose, mice were anesthetized with sodium pentobarbital (100 mg/kg). Pancreas, gastrocnemius muscle, liver and lung were taken and frozen immediately in liquid nitrogen and stored at −80 °C.

To determine the chronic effect of HSP90 inhibition, animals were treated for 15 days. On the first day of the study, plasma samples were obtained following a 2-h fast via tail bleeding. Plasma glucose concentrations were determined using a glucose meter (Ascensia Elite, Bayer Corp., Mishawaka, IN), and samples were collected in tubes (Microvette CB300, Aktiengesellschaft & Co., Numbrecht, Germany) containing EDTA to prevent clotting. Tubes were kept on ice and plasma portions of the blood samples were obtained by centrifugation at 10,000*g* for 10 min at 4 °C and stored at −80 °C.

Animals were randomly assigned into vehicle and compound groups (n = 7/group) with the means of plasma glucose levels and body weights matched among the groups. Animals were then dosed via tail vein injection (5 ml/kg) with vehicle (saline) or AUY922 at 5 or 15 mg/kg. The animals were dosed three times a week (on Monday, Wednesday and Friday) with either vehicle or the compound in vehicle. On the last day (day 16) of the study, 1 day after the last dose, animals were anesthetized and the tissues were collected. Plasma samples were collected via cardiac puncture. All the samples were stored at −80 °C.

2.3. OGTT and ITT

The insulin tolerance test (ITT) was performed using 0.8 U/kg Vetsulin (Intervet Inc., Millsboro, DE; 2 U/ml saline with 0.1% BSA, intraperitoneal injection at 4 ml/kg). Blood glucose levels were measured at 30, 60, and 120 min after the insulin injection. The oral glucose tolerance test (OGTT) was performed using 20% glucose at 5 ml/kg. Plasma glucose concentrations were measured before the OGTT (0 min) and at 30, 60 and 120 min after the glucose challenge.

2.4. Statistics

Data are reported as the means ± SEM. Statistical analysis was performed using a two-tailed and non-paired Student's *t*-test. Statistical significance was accepted at the level of $p < 0.05$.

3. Results

3.1. Establishment of HSF1 activation by HSP90 inhibitors

We used primary mouse skeletal myoblasts as a cell culture model to dissect the HSF1 pathway and its effects on insulin signaling. Extremely low levels of HSP70 protein expression under normal growth conditions reflected low basal HSF1 activity, and afforded a large window of activation as demonstrated with small molecule HSP90 inhibitors (Fig. 1A and B). Unlike the molecular derivatives of geldanamycin (a benzoquinone ansamycin antibiotic), such as 17-AAG, AUY922 is a novel resorcinylic isoxazole amide molecule that potently inhibits HSP90 with a half maximal inhibitory effect (Kd) of 1.7 nmol/L [14]. Thus, with at least two distinct structural classes of HSP90 inhibitors, we were able to pharmacologically reconstitute the HSF1-dependent stress response as determined by induction of HSP70 gene expression.

3.2. Cytoprotection and metabolic signaling transduction effects of HSP90 inhibitors in cells

To assess the cytoprotective potential of HSP90 inhibitors, two models of cellular stress were employed; (i) an acute muscle cell cytotoxic model using mouse primary myotubes, and (ii) a cytokine-induced inflammatory model in the pancreatic β-cell line,

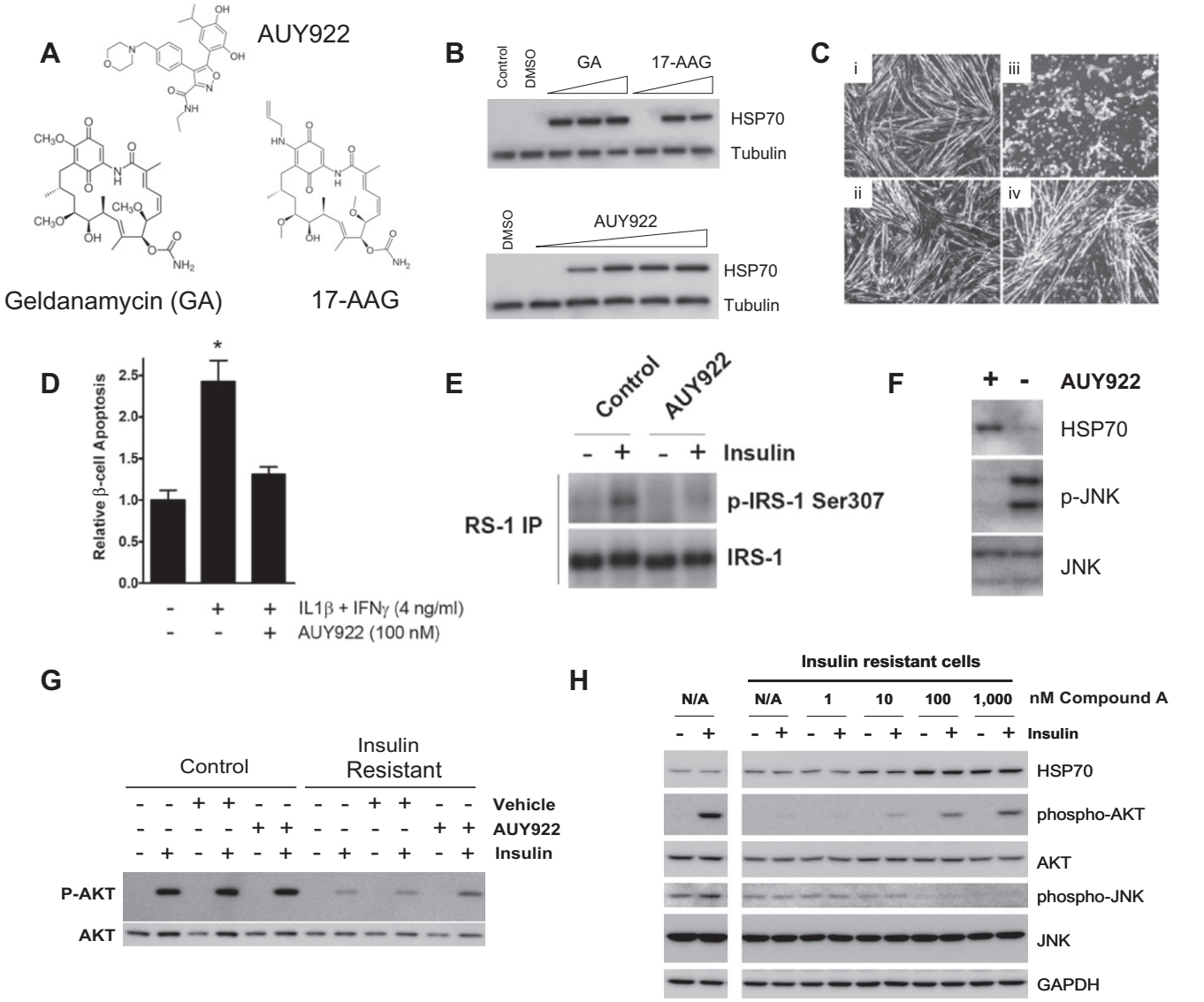

Fig. 1. HSP90 inhibitors are cytoprotective, and improve cellular metabolic signaling. (A,B) HSP90 inhibitors induce expression of HSP70. Primary mouse myotubes were exposed to increasing concentrations of the HSP90 inhibitors. Specifically, 100 nM, 1 μM, and 10 μM Geldanamycin (GA) and 17-AAG were added to cells, and 1 nM, 10 nM, 100 nM, 1 μM and 10 μM AUY922 was added for 5 h. Cell lysates were immunoblotted for HSP70. The data are representative of 3 independent experiments. (C) Primary mouse myotubes were either untreated (i) or treated with 10 nM AUY922 (ii), 1 nM dexamethasone (iii), or a combination of both (iv). After treatment for 18 h the cells were observed under a microscope. The data are mean ± SEM of 3 independent experiments. (D) INS-1E cells were incubated in the absence or presence of 100 nM AUY922 for 18 h and assayed for induction of apoptosis. The data are mean ± SEM of 3 independent experiments; $^{*}p < 0.001$. (E–H) C2C12 cells were made insulin resistant by exposure to a cocktail of dexamethasone, glucose and insulin for 3 days and were then treated with 100 nM AUY922 or indicated concentrations of compound A for 2 h prior to an acute (15 min) insulin challenge. (G,H) Insulin sensitivity was determined as a function of AKT phosphorylation (P-AKT). (E) Immunoprecipitation of IRS-1 was followed by immunoblotting for IRS-1 phospho-serine (upper panel) and total IRS-1 immunoreactive protein (lower panel) to determine effects of AUY922.

INS-1E. The myocyte cytotoxic model was established by exposing cells to the glucocorticoid receptor agonist dexamethasone for 18 h (Fig. 1C), (iii) resulting in a significant degree of myotube contraction and cell death. When co-treated with 10 nM AUY922, these cells were completely refractive to the cytotoxic effect of dexamethasone (Fig. 1C) (iv). In INS-1E cells, the combination of IL-1β and IFNγ in the presence of 11 mM glucose induced a 2.5-fold increase in apoptosis (Fig. 1D), which was attenuated by over 80% in the presence of AUY922. These two surrogate cell models of stress-induced cytotoxicity effectively revealed the cytoprotective effects of HSP90 inhibitors, consistent with known attributes of the HSF1 stress response pathway [15].

Insulin resistance is a hallmark of type 2 diabetes, typified at the molecular level by suppressed activation of the insulin signaling pathway at the level of the insulin receptor, IRS1/2, PI3K and AKT [16]. We created a state of insulin resistance in C2C12 muscle cells by exposing them to high levels of glucose, insulin and dexamethasone for three days. This resulted in significant attenuation of insulin-induced AKT phosphorylation, as observed *in vivo* by glucotoxicity, and this served as a useful cell-based model to assess the insulin sensitizing properties of AUY922 (Fig. 1E–H). Exposure of insulin resistant cells to AUY922 for 2 h resulted in restored sensitivity to insulin, as demonstrated by augmented insulin-induced AKT phosphorylation (Fig. 1G). Furthermore, this improvement in insulin sensitivity correlated with elevated HSP70 levels, and notably a significant attenuation in JNK phosphorylation levels (Fig. 1F). Indeed, a correlation between HSP70 and JNK phosphorylation is precedented by published reports that show a direct inhibitory interaction of HSP70 on JNK activity [17,18]. Moreover, we showed that AUY922 reduced IRS-1 phospho-serine levels, in accordance

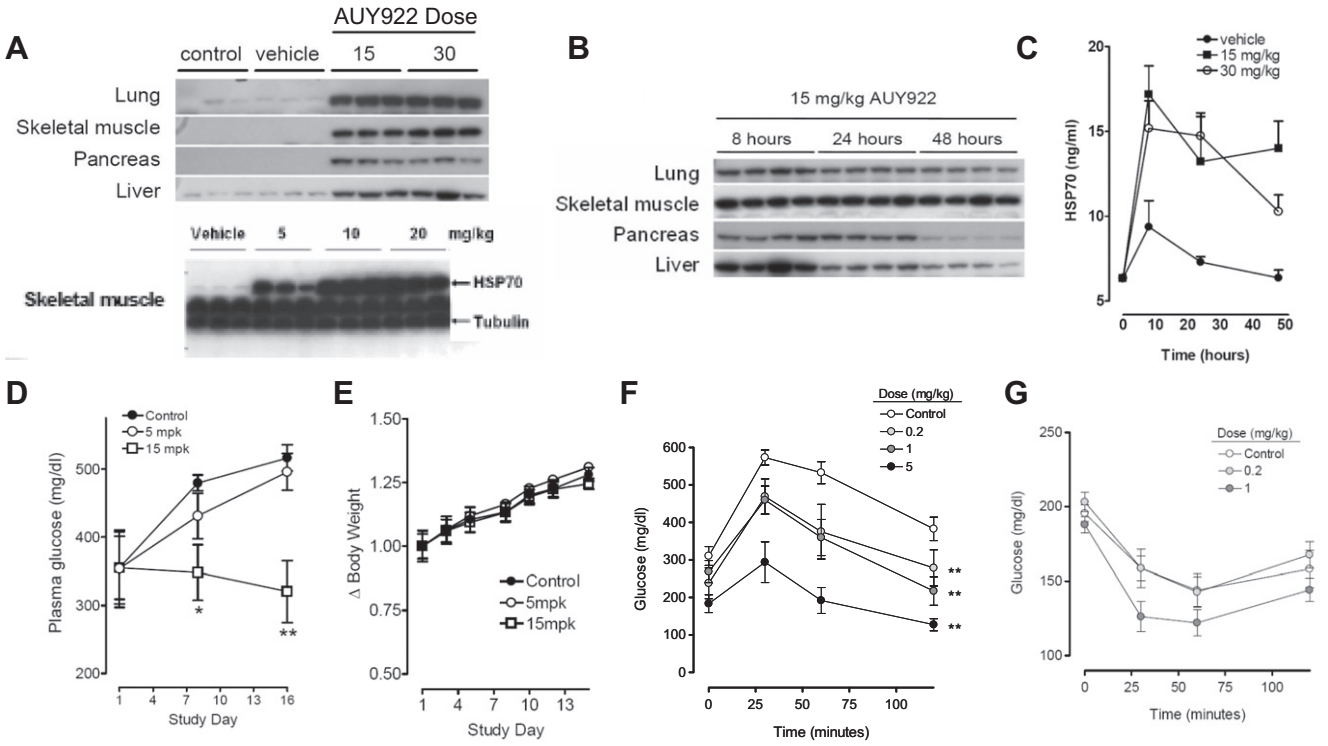

Fig. 2. HSP90 inhibitors robustly regulate glucose metabolism in diabetic mouse models. (A–C) Dose response and time-course of intravenous administration of AUY922 in various tissues and plasma in *db*/*db* diabetic mice using HSP70 as a pharmacodynamic biomarker. (A) Samples collected at 24 h after dosing. Each lane represents a mouse within the indicated treatment group. (B) Time-course of HSP70 expression in multiple tissues following a single intravenous dose of 15 mg/kg AUY922. (D,E) Effect of chronic treatment of *db*/*db* mice starting at 7 weeks of age. Mice were dosed by IV injection 3 times per week and fasting glucose levels were measured 2 to 3 days following the previous dose ($^{*}p < 0.01$; $^{**}p < 0.005$). Statistical significance was determined using a non-paired *t*-test. (F,G) Diet-induced obese (DIO) mice were dosed with compound A by gavage 3 times per week for 15 days. On day 16, an oral glucose tolerance test (OGTT) or an insulin tolerance test (ITT) was performed as described in Section 2.

with increased HSP70 expression and inhibition of JNK activity (Fig. 1E). These cell pharmacological features of AUY922 imply that HSP90 inhibition restores insulin sensitivity in muscle cells by inhibiting JNK-mediated insulin resistance. To confirm that these effects were not idiosyncratic responses of AUY922, we demonstrated the same signaling effects with a structurally distinct compound (Fig. 1H).

3.3. In vivo anti-diabetic effects of HSP90 inhibitors

As prerequisite to investigating *in vivo* efficacy, we characterized the pharmacodynamic properties of AUY922 following intravenous administration in 7 week old *db*/*db* mice, focusing on HSP70 protein levels as a biomarker of HSF1 activation. Mice were treated with a single dose of AUY922 at either 15 or 30 mg/kg (or vehicle only). After 24 h exposure, HSP70 levels were measured in multiple tissues, including lung, skeletal muscle, liver, pancreas and plasma (Fig. 2A and C). HSP70 levels were significantly elevated in each tissue at both concentrations of compound implying maximal activation of HSF1 was attained with 15 mg/kg. In addition, a time course of HSP70 elevation in various tissues showed a sustained activation beyond 48 h post-dose in lung and skeletal muscle, and to a lesser degree in the liver (Fig. 2B). In contrast, HSP70 levels appeared to normalize in the pancreas between 24 and 48 h post-dose.

We then performed a two week chronic treatment study in 7 week old *db*/*db* mice using a dosing regimen of 3 intravenous injections of AUY922 (5 and 15 mg/kg) per week. We measured fasting glucose levels 2–3 days following the previous dose, moments prior to the next dose of AUY922 in order to ensure that the effect on plasma glucose levels was due to improved metabolism rather than an acute response to drug. On day 16, the 15 mg/kg group displayed a 48% lower fasting plasma glucose level compared with the vehicle treated group, revealing a robust and sustained efficacy that lasted well beyond the plasma half-life of AUY922 at approximately 10 h [19] (Fig. 2D). In contrast, the 5 mg/kg group showed no significant difference in glucose levels, indicating clear dose-dependency. Body weight was unaffected by AUY922 treatment (Fig. 2E).

Using compound A, another HSP90 inhibitor with oral bioavailability in the insulin resistant diet-induced obese (DIO) mouse, we observed an improvement in glucose excursion in the context of an oral glucose tolerance test (OGTT) following intermittent dosing for 15 days. An improvement in insulin sensitivity was also determined by an insulin tolerance test (ITT) on day 16 (Fig. 2F and G). These effects in the diabetic *db*/*db* model and the insulin resistant DIO model strongly support the therapeutic potential for HSP90 inhibitors as anti-diabetic drugs, and support the role of the HSF1 pathway as a mediator of anti-inflammation and increased insulin sensitivity leading to improved glucose metabolism.

4. Discussion

Though circumstantial, the coincidences of reduced skeletal muscle HSP70 levels in type 2 diabetes patients [1], and the correlation between HSP70 levels and insulin sensitivity in a separate study [2], add support to the potential clinical translation of our observations that HSF1-induced HSP70 expression mediates an insulin sensitizing effect in skeletal muscle. Moreover, transgenically engineered mice over-expressing the HSP70 gene in skeletal muscle are protected against diet-induced insulin resistance [20], consistent with our observations using a pharmacological

approach to augment HSP70 levels in skeletal muscle. In another study, heat treatment alone improved glucose tolerance and attenuated insulin resistance in skeletal muscle of rats fed on a high-fat diet [21], further linking heat shock response to insulin sensitivity. These studies provide independent support for a functional role of HSF1 in muscle insulin sensitivity, and in turn glucose control. The observed cytoprotective effects of AUY922 on INS-1 cells exposed to inflammatory cytokines provide additional potential for disease modification, specifically in regard to the chronic effects of inflammatory cytokines and glucolipotoxicity on pancreatic beta cell function in many type 2 diabetes patients. Mechanistically, this observation is unsurprising, given the wealth of published data showing cytoprotective effects of the HSF1 mediated protein chaperone pathway in cell types ranging from neurons [22] to cardiomyocytes [23].

In summary, these data provide evidence that pharmacological effects of HSP90 inhibitors on metabolic endpoints represent a novel therapeutic pathway for the regulation of glucose metabolism and potential treatment of type 2 diabetes. Corroboration of our insights may be drawn from a diverse array of published genetic, molecular and clinical data that overall conveys a convincing argument for the concept of stress response pathways mediating metabolic control at the cellular and physiological level. More work remains to be done in order to convincingly prove that the effects of HSP90 inhibitors observed here are specifically mediated by HSF1 and HSP70. This is the first reported evidence that the HSP90 inhibitor class of small molecules has therapeutic potential for type 2 diabetes and other glycemic conditions.

Acknowledgments

We thank Tim Machajewski, Zhenhai Gao, and Michael Ruggard-Jensen for characterization of HSP90 inhibitor compounds, including AUY922 and Jinsheng Liang for *in vivo* study support.

References

[1] I. Kurucz, A. Morva, A. Vaag, K.F. Eriksson, X. Huang, L. Groop, L. Koranyi1, Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance, Diabetes 51 (2002) 1102–1109.

[2] C.R. Bruce, A.L. Carey, J.A. Hawley, M.A. Febbraio, Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes, Diabetes 52 (2003) 2338–2345.

[3] G. Liu, C.M. Rondinone, JNK: bridging the insulin signalling and inflammatory pathway, Curr. Opin. Investig. Drugs 6 (2005) 979–987.

[4] H. Kaneto, D. Kawamori, Y. Nakatani, S.I. Gorogawa, A. Matsuoka, Oxidative stress and the JNK pathway as a potential therapeutic target for diabetes, Drug News Perspect. 17 (2004) 447–453.

[5] G.S. Hotamisligil, Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes, Diabetes 54 (2005) S73–S78.

[6] H. Cho, S.C. Black, D. Looper, M. Shi, D. Kelly-Sullivan, S. Timofeevski, K. Siegel, X.-H. Yu, S.R. McDonnel, P. Chen, J. Yuie, K.M. Ogilvie, M. Fraser, C.P. Briscoe, Pharmacological characterization of a small molecule inhibitor of c-Jun kinase, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E1142–E1151.

[7] J. Hirosumi, G. Tuncman, L. Chang, C.Z. Gorgun, K.T. Uysal, K. Maeda, M. Karin, G.S. Hotamisligil, A central role for KNK in obesity and insulin resistance, Nature 420 (2002) 333–336.

[8] V. Aguirre, T. Uchida, L. Yenush, R. Davis, M.F. White, The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser307, J. Biol. Chem. 275 (2000) 9047–9054.

[9] T.L. Hilder, J.C.L. Tou, R.E. Grindeland, C.E. Wade, L.M. Graves, Phosphorylation of insulin receptor substrate-1 serine 307 correlates with JNK activity in atrophic skeletal muscle, FEBS Lett. 553 (2003) 63–67.

[10] G. Tuncman, J. Hirosumi, G. Solinas, L. Chang, M. Karin, G.S. Hotamisligil, Functional *in vivo* interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance, Proc. Natl. Acad. Sci. 103 (2006) 10741–10746.

[11] M.F. McCarty, Induction of heat shock proteins may combat insulin resistance, Med. Hypotheses 66 (2006) 527–534.

[12] M. Schulz-Raffelt, M. Lodha, M. Schroda, Heat shock factor 1 is a key regulator of the stress response in *Chlamydomonas*, Plant J. 52 (2007) 286–295.

[13] J. Zou, Y. Guo, T. Guettouche, D.F. Smith, R. Voellmy, Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1, Cell 94 (1998) 471–480.

[14] S.A. Eccles, A. Massey, F.I. Raynaud, S.Y. Sharp, G. Box, M. Valenti, L. Patterson, A.H. Brandon, S. Gowan, F. Boxall, W. Aherne, M. Rowlands, A. Hayes, V. Martins, F. Urban, K. Boxall, C. Prodromou, L. Pearl, K. James, T.P. Matthews, K.-M. Cheung, A. Kalusa, K. Jones, E. McDonald, X. Barril, P.A. Brough, J.E. Cansfield, B. Dymock, M.J. Drysdale, H. Finch, R. Howes, R.E. Hubbard, A. Surgenor, P. Webb, M. Wood, L. Wright, P. Workman, NVP-AUY922; a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis, Cancer Res. 68 (2008) 2850–2860.

[15] J. Yang, K. Bridges, K.Y. Chen, A.Y.C. Liu, Riluzole increases the amount of latent HSF1 for an amplified heat shock response abd cytoprotection, PLoS One 3 (2008) e2864–e2875.

[16] E. Tomas, Y-S. Lin, Z. Dagher, A. Saha, Z. Luo, Y. Ido, N.B. Ruderman, Hyperglycemia and insulin resistance: possible mechanisms, Ann. N.Y. Acad. Sci. 967 (2002) 43–51.

[17] A.S. Bienemann, Y.B. Lee, J. Howarth, J.B. Uney, HSP70 supresses apoptosis in sympathetic neurones by preventing the activation of c-jun, J. Neurochem. 104 (2008) 271–278.

[18] H.-S. Park, J.-S. Lee, S.H. Huh, J.-S. Seo, E.-J. Choi, HSP72 functions as a natural inhibitory protein of c-Jun N-terminal kinase, EMBO 20 (2001) 446–456.

[19] M.R. Jensen, J. Schopefer, T. Radimerski, A. Massey, C.T. Guy, J. Brueggen, C. Quadt, A. Buckler, R. Cozens, M.J. Drysdale, C. Garcia-Echeverria, P. Chene, NVP-AUY922: a small molecule HSP90 inhibitor with potent antitumor activity in preclinical breast cancer models, Breast Cancer Res. 10 (2008) R33–R45.

[20] J. Chung, A.-K. Nguyen, D.C. Henstridge, A.G. Holmes, M.H.S. Chan, J.L. Mesa, G.I. Lancaster, R.J. Southgate, C.R. Bruce, S.J. Duffy, I. Horvath, R. Mestril, M.J. Watt, P.L. Hooper, B.A. Kingwell, L. Vigh, A. Hevener, M.A. Febbraio, HSP72 protects against obesity-induced insulin resistance, Proc. Natl. Acad. Sci. 105 (2008) 1739–1744.

[21] A.A. Gupte, G.L. Bomhoff, R.H. Swerdlow, P.C. Geiger, Heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in rats fed a high-fat diet, Diabetes 58 (2009) 567–578.

[22] Z. Batulan, D.M. Taylor, R.J. Aarons, S. Minotti, M. Doroudchi, J. Nalbantoglu, H.D. Durham, Induction of multiple heat shock proteins and neuroprotection in a primary culture model of familial amyotrophic lateral sclerosis, Neurobiol. Dis. 24 (2006) 213–225.

[23] Y. Zou, W. Zhu, M. Sakamoto, Y. Qin, H. Akazawa, H. Toko, M. Mizukami, N. Takeda, T. Minamino, H. Takano, T. Nagai, A. Nakai, I. Komuro, Heat shock transcription factor 1 protects cardiomyocytes from ischemia/reperfusion injury, Circulation 108 (2003) 3024–3030.